

## The effect of high-intensity interval training on follistatin, irisin plasma levels and lipid profile in overweight and obese women

Naser Rostamzadeh 

Department of Physical Education, Farhangian  
University, Tehran, Iran.

Dara Latif Saifaddin 

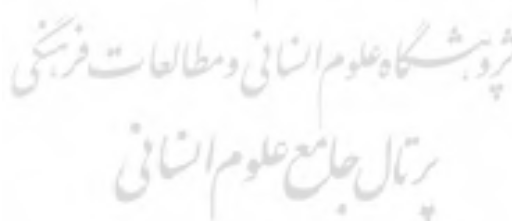
Department of Physical Education and Sport  
Sciences, University of Halabja, Kurdistan  
region, Iraq.

Saeed Abdi 

Department of Physical Education, Farhangian  
University, Tehran, Iran.

Saiwan Sirwan Muhammed 

Department of Physical Education and Sport  
Sciences, University of Halabja, Kurdistan  
region, Iraq..



Corresponding Author: ahmad.fasihi44@gmail.com

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### Abstract

**Purpose:** The aim of this study was to investigate the effect of high-intensity interval training on serum levels of irisin, follistatin, and metabolic status of overweight and obese women. **Methods:** The current research was a semi-experimental type with a pre and post-test design. 30 women with an average age of  $22.9 \pm 2.11$  years, weight  $82.45 \pm 6.1$  kg, BMI above 28 kg/m<sup>2</sup>) voluntarily participated and they were randomly divided into two groups of 15 people including HIIT (4 bouts of 60 seconds of running at an intensity of 80-90% HR<sub>max</sub> with a 4-minute rest in the first four weeks) and control (did not have a regular exercise program). Blood samples were taken 24 hours before the start of the training period and 48 hours after the end of the last session, to measure irisin, Follistatin, and metabolic status, by ELISA method. Then, an independent t-test was used to examine changes between groups, and a correlated t-test was used for intragroup comparison. Data analysis was done by SPSS version 25 software at a significance level of 0.05. **Results:** After 8 weeks of intervention, the HIIT group showed that HIIT exercises led to a significant increase in irisin( $p=0.25$ ) and follistatin( $p=0.12$ ), triglyceride ( $p=0.30$ ), and total cholesterol levels. ( $p=0.41$ ), LDL ( $p=0.14$ ), VLDL ( $p=0.17$ ), weight ( $p=0.31$ ), fat percentage ( $p=0.21$ ) and BMI ( $p=0.251$ ); And there was a significant increase in maximum oxygen consumption ( $p=0.11$ ). However, no significant change was observed in the amount of HDL ( $p=0.55$ ). **Conclusion:** According to the findings of the present study, in order to maintain or prevent the decrease in bone density of postmenopausal women, resistance exercises can be recommended.

**Keywords:** follistatin, irisin, lipid profile, obese women, interval training.

## Introduction

Considering that obesity and overweight are among the problems of today's societies, preventing and reducing it seems very necessary (Medina, Solé-Sedeno, Bach-Faig, & Aguilar-Martínez, 2021). Obesity is a global public health problem and a global epidemic of the 20th century (Koletzko et al., 2020). A large part of research has been done in order to search for preventive and therapeutic goals with a major focus on fat tissue and its different types (Poniedziałek-Czajkowska, Mierzyński, & Leszczyńska-Gorzelak, 2023). Currently, physical activity is recommended as the first line of treatment for obesity, because it causes weight loss (Headid III & Park, 2020). The fat mass improves the main metabolic and cardiovascular risk factors and increases cardio-respiratory fitness (Hamasaki, 2024). The mechanisms underlying the physical benefits of exercise in obesity management are partially known (Batrakoulis et al., 2022). During muscle contraction, myocytes act as a secretory organ and release hormones called myokines (Severinsen & Pedersen, 2020). Irisin is a member of the myokine family, which is secreted from skeletal muscle cells by breaking the FNDC5 protein (Hoffmann & Weigert, 2017). This myokine is released from skeletal muscles during physical activity and acts as a link between skeletal muscle and other organs and tissues. Also, some studies show that irisin is also secreted from fat tissue and cardiac muscle, and by helping to increase the expression of various metabolic enzymes and mediators, it inhibits fat accumulation. Preclinical studies showed that the release of irisin caused by exercise causes the browning of fat tissue and the improvement of glucose and lipid metabolism (Fox et al., 2018)]. Some animal and human studies have shown an increase in circulating irisin levels after exercise (Brenmoehl et al., 2014; Hecksteden et al., 2013). It has been reported that the level of irisin in people who exercise regularly is significantly higher than in sedentary people.

Follistatin is another myokine that is expressed and secreted in all tissues and especially by skeletal muscles. The most important function of this myokine is to inhibit the action of TGF- $\beta$  family proteins,

including myostatin. Inhibition of myostatin helps to hypertrophy skeletal muscle and reduce fat mass. Increasing the release of follistatin in the body acts as a protective mechanism for muscle mass and helps regulate body composition by catabolizing fat mass and burning excess fat and causes obesity or overweight to improve. In the presence of follistatin, myostatin is unable to bind to its receptor and thus its function is impaired. Researches have shown that performing sports exercises increase the amount of follistatin and the ratio of follistatin/myostatin and decrease the amount of myostatin. Also, in a study, Kalli et al. (1400) reported the lack of effect of aerobic exercises on follistatin levels of overweight people. Eisazadeh et al. They did not, and they stated that one of the reasons for this issue is the lengthening of the total combined training time and the fatigue caused by it.

HIIT training is a form of intense training in which bouts of intense activity are separated by active or passive recovery (Ben Abderrahman et al., 2013). These exercises increase the oxidation of fat and carbohydrates in the skeletal muscle and are a suitable stimulus for weight loss compared to aerobic exercises (Schoenmakers, Hettinga, & Reed, 2019). On the other hand, intense activity causes a significant increase in circulating hormones, and since HIIT exercises are part of this group of exercises, it is expected that these types of exercises can change the hormonal status (Ramos, Dalleck, Tjonna, Beetham, & Coombes, 2015). The results of previous researchers show that exercise is one of the most important factors affecting the expression and secretion of irisin (Wahl et al., 2013). The findings of Colpitts et al.'s research (2022) showed that HIIT exercises cause a greater increase in irisin levels in young subjects than continuous moderate intensity exercises (Colpitts, Rioux, Eadie, Brunt, & Sénéchal, 2022). Some studies also show that HIIT exercises have beneficial and even better effects than exercises (Atakan, Li, Koşar, Turnagöl, & Yan, 2021; Cipryan, Tschakert, & Hofmann, 2017). Interval training involves alternating periods of exercise and rest, although most researchers believe that HIIT exercises are the best Exercise is for health and weight loss, but there are still many questions regarding the effect of many

hormones. Therefore, research that examines the effect of high intensity interval training (HIIT) on irisin, follistatin and some glycemic indices is necessary (Torma et al., 2019). Therefore, this study was conducted with the aim of investigating the effect of high-intensity interval training on plasma levels of follistatin, irisin and lipid profile in overweight and obese women.

### **Methods**

The current research was a semi-experimental type with a pre-test and post-test design. The statistical sample of this research was made up of women referring to the sports clubs of Hamedan city, after informing and sending the call, finally, 30 women were selected from among the volunteers (age range 25-35 years). Then the subjects were They were randomly divided into two groups of 15 people who performed HIIT exercises for eight weeks (three sessions each week). Body mass index ( $28 \text{ kg/m}^2$  or higher) was included in the study including healthy, no history of taking drugs affecting lipid profile, no history of smoking, no history of cardiovascular disease, liver disease, or kidney disease. The exclusion criteria were: the subject's unwillingness to continue the exercises and absenteeism for more than two consecutive sessions.

### **Research implementation method**

First, the subjects were invited to participate in the briefing session to get familiar with the research steps. In this meeting, the subjects were introduced to the implementation of research plans and completed the consent forms for participating in the research. One week before the start of the training program, the subjects were familiarized with the correct way of performing techniques, movements and tests, and information such as age, height, weight, and body mass index (BMI) were evaluated and recorded. To measure the fat percentage, people were asked to sit straight and anatomically. Body fat percentage (BF%) was calculated by measuring the thickness of subcutaneous fat by caliper in three regions of chest, abdomen and thigh and using the three-point equation of Jackson Pollack. One day before the start of the

training program, all subjects were invited to attend the laboratory. In the laboratory, in order to determine the basic levels of irisin, follistatin, and lipid profile, ten milliliters of blood was taken from the brachial vein by a laboratory specialist. 24 hours later, the subjects of the experimental groups participated in eight weeks of HIIT exercises. At the end and 48 hours after the last training session, ten milliliters of blood were taken from the subjects of the experimental and control groups (who did not have any regular sports activities during this period). After clotting in the laboratory environment, it was centrifuged for 10 minutes at a speed of 3000 revolutions per minute in order to separate the serum. Both stages of blood sampling were done between eight and nine in the morning and after 12 hours of fasting .

After collecting data, Shapiro Wilk test was used for statistical analysis to determine the normal state of data distribution, Levene's test was used to check the homogeneity of variances, and descriptive statistics were used to calculate centrality and dispersion indices. After ensuring the normality of the data, an independent t-test was used to compare the average parameters, and a paired t-test was used to evaluate the intra-group data between the pre-test and post-test of the training group. Statistical analysis was done at the statistical level of  $P \leq 0.05$  and data analysis was done using SPSS version 23 software.

## Results

In the table of the descriptive report and the results of inferential statistics regarding the comparison of the investigated indicators, it is presented. (Table 1)

**Table 1:** Anthropometric characteristics of subjects

<b>Groups Variables</b>	<b>Exercise Mean + SD</b>	<b>Control Mean + SD</b>
<b>Age (Year)</b>	25.56±5.11	26.86±6.21
<b>Weight (kg)</b>	81.14±7.4	80.21±5.15
<b>Height (cm)</b>	152.11±8.5	153.21±7.24
<b>BMD (kg/m<sup>2</sup>)</b>	30.17±0.88	30.07±0.38

In the outgroup comparison, the results of the independent t test showed that; After 8 weeks of HIIT, follistatin ( $p=0.022$ ) and irisin ( $p=0.012$ ) values increased significantly in the experimental group, but no significant change was observed in the control group. In addition, the intra-group evaluation of the data showed that the post-test values of the HIIT training group in follistatin ( $p=0.017$ ) and irisin ( $p=0.011$ ) variables increased significantly compared to the pre-test. Also, in the outgroup comparison, the results of the independent t-test showed that; After 8 weeks of HIIT, serum levels of triglyceride ( $p=0.046$ ), HDL ( $p=0.022$ ), total cholesterol ( $p=0.032$ ), and BMI ( $p=0.011$ ) of the participants They decreased significantly; while no significant change was observed in the control group. In the intra-group comparison, the results of the correlated t-test showed that; After 8 weeks of HIIT, the serum triglyceride ( $p=0.037$ ), total cholesterol ( $p=0.026$ ), HDL ( $p=0.047$ ) and BMI ( $p=0.044$ ) values of the participants after the ratio test They were significantly reduced in the pre-test. (Table 2)

**Table2.** Comparison of the mean and standard deviation of dependent variables before and after 10 weeks of swimming training in four groups using analysis of covariance and paired t-test

Variable	group	stage		dependent t results
		pre-test Mean + SD	post-test Mean + SD	p-value
Follistatin(ng/ml)	Exercise	28.50±3.41	34.52±5.40	0.017*
	Control	29.32±5.61	39.42±5.51	0.742
	independent t test (p-value)	0.534	0.022*	
Irisin, (ng/ml)	Exercise	4.36±0.81	6.14±1.40	0.011*
	Control	4.32±0.61	4.12±0.51	0.654
	independent t test (p-value)	0.751	0.016*	
TG (mg/dl)	Exercise	148.50±39.81	122.12±22.40	0.037*
	Control	150.32±30.61	150.12±31.51	0.829
	independent t test (p-value)	0.624	0.046*	
HDL-C (mg/ dl)	Exercise	35.25±13.55	41.62 ± 9.03	0.047*
	Control	34.27±12.58	34.22 ± 10.53	0.531
	independent t test (p-value)	0.591	0.022*	
LDL-C (mg/dl)	Exercise	129.65±31.53	125.54±17.13	0.038
	Control	127.26±32.31	125.75±22.15	0.128
	independent t test (p-value)	0.765	0.842	
TC (mg/dl)	Exercise	182.28 ± 43.44	140.75±22.53	0.026*
	Control	181.25 ± 42.35	183.50±40.53	0.502
	independent t test (p-value)	0.751	0.032*	
BMI (kg/m2)	Exercise	30.17±0.88	28.01±0.93	0.044*
	Control	30.07±0.38	30.11±0.22	0.982
	independent t test (p-value)	0.533	0.011*	



## Discussion

The results of the present study showed that HIIT exercises increase the plasma levels of irisin in overweight and obese women. These results are consistent with the results of some researches. In past studies, it has been shown that high-intensity interval training increases irisin in young and healthy men and young and healthy adults (Haghighi, Hajinia, Askari, Abbasian, & Goldfied, 2022). Also, aerobic exercise increases blood irisin levels in young and healthy men (Kraemer, Shockett, Webb, Shah, & Castracane, 2014). It has also been shown that high-intensity interval training increases irisin in young and healthy men and young and healthy adults (Jandova et al., 2021). Poutafkand et al 2020, investigated the plasma level of irisin in two activities with different intensity of 40 and 80% of VO<sub>2</sub>Max (Poutafkand, Marefati, & Taherichadorneshin, 2020). The results showed that the response of plasma irisin to intense exercise is higher and its value is higher immediately after exercise. The findings of a meta-analysis study show that the concentration of irisin in the circulation in adults increases significantly immediately after an acute period of exercise, which is in line with the results of this research (Kazeminasab, Sadeghi, & Afshari-Safavi, 2022). Irisin is a hormone that is made of protein. FNDC5 is isolated and encoded by an exercise-stimulating gene called FNDC1 (Motahari Rad, Bijeh, Attarzadeh Hosseini, & Raouf Saeb, 2021). Irisin causes browning of white adipose tissue and stimulates the expression of metabolic genes that play a role in oxidative phosphorylation and increasing energy consumption (Hecksteden et al., 2013). It is possible that the reduction of ATP, the metabolic requirement, is the primary signal that triggers the release of irisin to defend muscle ATP homeostasis during exercise (Momenzadeh et al., 2022). Although irisin is known as an exercise-induced myokine, some studies show different results. A decrease in the level of blood irisin was also observed after high-intensity interval training, Crossfit training and resistance training, which was inconsistent with the present study. The contradiction of these studies may be due to the difference

between the weight of the load and the duration of the physical exercise and the age of the participants.

Another result of this research was a significant increase in follistatin levels in response to intense interval training. The results of some research show that performing physical activities increases the levels of follistatin as a positive regulator of the muscle growth factor. The research results of Aghabigi et al. (2020) show a significant increase in follistatin levels after eight weeks of endurance training in women. showed. By investigating the effect of eight weeks of aerobic exercise on follistatin and myostatin in overweight women, Shojiei et al. (2018) reported a significant increase in follistatin levels. In the research of Taheri et al. (2014) there was also a significant increase in levels Follistatin was reported in response to ten weeks of central muscle training in elderly women. The duration and intensity of training is one of the regulating factors of muscle growth, especially follistatin, in such a way that aerobic exercises also improve the structure of muscle tissue by increasing the amounts of follistatin. increase in oxidative stress and inflammation by disrupting the regulation of myokines, play a key role in muscle changes. The antioxidant and anti-inflammatory effects of sports activities, especially interval training, have been accepted. The results of some studies show that exercise By increasing mitochondrial biogenesis, reducing oxidative damage, reducing chronic inflammation and improving myokine profile, it improves muscle structure and function. Based on this, it can be concluded that the increase in follistatin and irisin levels can be due to the improvement of the muscle tissue structure in the subjects, as well as the anti-fatigue and anti-inflammatory benefits of HIIT exercises.

The results of the present study showed that intense interval training caused a significant decrease in glycemic indices such as triglyceride, total cholesterol, body mass index and HDL plasma of the subjects. It seems that physical activity increases the ability of skeletal muscle to use fat instead of glycogen and therefore reduces the level of blood lipids. The mechanisms involved in this process include an increase in the amount of lecithin cholesterol acyltransferase (LCAT) enzymes (an

enzyme involved in the transfer of esters to HDL cholesterol) and lipoprotein lipase and a decrease in cholesterol ester transfer protein (CETP) (an enzyme responsible for the transfer of HDL cholesterol to other lipoproteins). The change in the amount of enzymes by improving the ability of muscle fibers to oxidize plasma fatty acids causes a decrease in HDL cholesterol and triglycerides. BMI and thus be effective in reducing the fat percentage of obese and overweight women. Therefore, HIIT exercises can be used as a safe method to have favorable effects on some body composition factors .

The current research has limitations that need to be noted. First, the small number of subjects was one of the limitations of this study. Also, the small number of groups is one of the other limitations of this research that can affect the obtained results and should be taken into consideration in future research.

### Conclusion

Performing HIIT, repeated three sessions a week for 8 weeks, as a non-drug and effective method, can reduce the plasma levels of follistatin and irisin in overweight and obese women, and subsequently, lipid profile and body composition of improve them.

### Conflict of interest

The authors hereby declare that there is no conflict of interest in the present study.

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### ORCID

Naser Rostamzadeh	 <a href="https://orcid.org/">https://orcid.org/</a>
Dara Latif Saifaddin	 <a href="https://orcid.org/">https://orcid.org/</a>
Saeed Abdi	 <a href="https://orcid.org/">https://orcid.org/</a>
Saiwan Sirwan Muhammed	 <a href="https://orcid.org/">https://orcid.org/</a>

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Corresponding Author: ahmad.fasihi44@gmail.com

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